REMARKS

Claims 1-12 are pending in this application. Claims 1-12 have been rejected. Claims 4, 6-9, 11 and 12 have been amended. Claim 1-3 and 5 have been canceled. No new matter has been added by this amendment. Reconsideration is respectfully requested in light of these amendments and the following remarks.

I. New Grounds for Rejection

Due to new grounds for rejection set forth under 35 U.S.C. §102 and §103, the Examiner has withdrawn allowance of claim 8.

II. Acknowledgment of Previous Response to Arguments

The Examiner has acknowledged that the previous amendment, submitted on 16 October 2003, successfully overcame the rejection of claims 1, 2, 4, 5, 7, and 9-12 under 35 U.S.C. § 102.

III. Objection to Claims

Claim 3 has been objected to for failing to further limit claim 2 from which it depends and further objected to under 37 CFR 1.75 as being a substantial duplicate of claim 2. Applicant has canceled claim 3. Withdrawal of this objection is respectfully requested.

IV. Rejection of Claims under 35 U.S.C. §112

The Examiner has rejected claims 1-5, 7, and 9-12 under 35 U.S.C. § 112, first paragraph as containing subject matter which

was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors at the time the application was filed had possession of the claimed invention. The Examiner suggests that claims 1-5 and 7, drawn to the genus of non-classical nuclear localization signals (NLSs) that do not interact with importin alpha and importin beta can be interpreted to mean that the non-classical NLS cannot bind to importin alpha of an importin alpha/beta complex but can still bind to importin beta. The Examiner further suggests that the central issue is whether Applicant has disclosed a number of species which is representative of the claimed genus. The Examiner suggests that the genus of nonclassical NLSs is of indeterminate size because it includes more than the M9, KNS, and HNS sequences disclosed in the specification as said genus includes sequences that localize to the nucleus by affinity for nuclear structures (*e.g.*, zinc finger proteins like ZNF74, κBα, 53BP2, GABPβ, or Histone H1) and comprises a high degree of variability. The Examiner further suggested that while the specification teaches nonclassical NLSs such as M9 and HNS, which enter the nucleus by interacting with transportin, M9 and HNS show no homology (Fan and Steitz (1998) *Proc. Natl. Acad. Sci. USA* 95:15293-98) and the specification fails to teach what structural feature these proteins have in common which allows them to utilize the same nuclear import pathway. It is further suggested that the specification fails to provide any known or disclosed correlation between structure and function that could be considered to be an adequate description of

relevant identifying characteristics of the genus of nonclassical NLSs. Applicant respectfully traverses this rejection.

Compliance with written description requirement of 35 U.S.C. 112, first paragraph, may be shown by any description of sufficient, relevant, identifying characteristics so long as a person skilled in the art would recognize that the inventor had possession of the claimed invention. For some biomolecules, examples of identifying characteristics include a sequence, structure, binding affinity, binding specificity, molecular weight, and length. Although structural formulas provide a convenient method of demonstrating possession of specific molecules, other identifying characteristics or combinations of characteristics may demonstrate the requisite possession. See MPEP §2163.

Accordingly, in an effort to advance the prosecution of the present application, Applicant has canceled claims 1-3 and amended claims 4, 7-9, 11, and 12 to clearly recite identifying binding specificity characteristics of the nonclassical NLSs of the present invention. Claims 4, 7 and 8 have been amended to clarify that the nonclassical NLSs do not bind to either importin-α or importin-β as indicated on page 8, lines 12-14, which states the nonclassical NLS do not interact with proteins such as importin-α and importin-β. Claims 4, 7, 9, 11, and 12 have also been amended to recite that the NLSs interact with transportin to mediate nuclear pore targeting and import of molecules into the nucleus of the cells. Support for this amendment is found on page 8, lines 26-28. In light of these amendments, claim 5 has been canceled.

As amended, the claimed invention encompasses M9 and HNS, which enter the nucleus by interacting with transportin and not

importin-α or importin-β. At the outset, Applicant respectfully disagrees with the Examiner's interpretation of the teachings of Fan and Steitz (1998) in suggesting that M9 and HNS show no homology. What this reference, which was filed within four months of the filing date of the instant application, does in fact show is that at or around the time of filing of the instant application, it could be readily recognized by one of skill that a structural and functional relationship indeed existed between nonclassical NLSs of the genus encompassing HNS and M9. See, *e.g.*, Figure 6B of Fan and Steitz (1998) which shows structural features in common between HNS and M9; a conserved NLS motif of QXXXFXPMXXXXXXGXS, which is high in glycine and serine content. Applicant believes that the identifying functional characteristics (*i.e.*, interacting with transportin, rather than importin-α or importin-β, to mediate nuclear import of the protein) of nonclassical NLSs provided by the specification in combination with art-recognized structural features of the same (*i.e.*, a conserved NLS motif of QXXXFXPMXXXXXXGXS, which is high in glycine and serine content) meet the written description requirement of 35 U.S.C. 112, first paragraph. Reconsideration and withdrawal of the rejections under 35 U.S.C. §112, first paragraph is therefore respectfully requested.

V. Claim rejections under 35 USC 102

As Applicant has canceled claims 1-3 and 5, arguments to rejections under 35 U.S.C. §102 will be directed to claims 4, 6, 7 and 9-12.

Claims 1-6 have been rejected under 35 U.S.C. §102 (b) as being anticipated by Michael et al. (1995) *Cell* 82:415-422 in its teaching of a 38 amino acid peptide identical of SEQ ID NO:3 of the instant invention, wherein said peptide functions as a NLS of a fusion partner. Applicant respectfully traverses this rejection.

MPEP 2131 states "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." Verdegaal Bros. v. Union Oil Co. of California, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987).

While Michael et al. (1995) teach a 38 amino acid domain of A1 which is identical to SEQ ID NO:3 of the instant invention and functions to transport fusion proteins into the nucleus, this reference does not teach each and every element of amended claims 4 and 6, *i.e.* that the NLS interacts with transportin, rather than importin-α or importin-β, to mediate nuclear pore targeting and import of molecules into the nucleus of cells. Thus, in accordance with MPEP 2131, this reference does not anticipate pending claims 4 and 6 under 35 U.S.C. §102 (b) and withdrawal of this rejection is respectfully requested.

Claims 1-5 are rejected under 35 U.S.C. §102 (b) as being anticipated by Fan and Steitz (Dec. 1998) *Proc. Natl. Acad. Sci. USA* 95:15293-15298 as evidenced by Gallouzi et al. (Nov. 2001) *Science* 294:1895-901. The Examiner suggests that Fan and Steitz teach HNS for nuclear localization of a fusion protein and Gallouzi teaches that HNS interacts with transportin 2. Applicant respectfully traverses this rejection.

Fan and Steitz (1998) teach that HNS can import a fusion protein into the nucleus, however, this reference does not teach that HNS interacts with transportin, and not importin-α or importin-β, to mediate nuclear pore targeting and import of molecules into the nucleus of cells. Applicant respectfully disagrees with the Examiner's reasoning that because Gallouzi et al. (2001) demonstrate an interaction with transportin 2 that said interaction implies nuclear import. In fact, Gallouzi et al. only teach export of HNS via transportin 2. Thus, it cannot be assumed that the import of the fusion protein of Fan and Steitz (1998) is via transportin 2 as evidenced by Gallouzi et al.; the mechanism of HNS nuclear import may have been independent of transportin 2. Thus, in accordance with MPEP 2131, the reference of Fan and Steitz (1998) either alone or in view of Gallouzi et al. fails to anticipate pending claim 4 under 35 U.S.C. §102 (b) and withdrawal of this rejection is respectfully requested.

Claims 1 and 4 are rejected under 35 U.S.C. §102 (b) as being anticipated by Michael et al. (1997) *EMBO J.* 16:3587-3598 in its teaching of KNS for directing nuclear localization of a fusion protein. Applicant respectfully traverses this rejection.

Michael et al. (1997) teach KNS sequences capable of directing nuclear localization of a fusion protein. This reference does not teach each and every element of amended claim 4, *i.e.* that KNS interacts with transportin, rather than importin-α or importin-β, to mediate nuclear pore targeting and import of molecules into the nucleus of cells. Thus, in accordance with MPEP 2131, this

reference does not anticipate pending claim 4 under 35 U.S.C. §102 (b) and withdrawal of this rejection is respectfully requested.

Claims 1, 4 and 7 are rejected under 35 U.S.C. §102 (a) as being anticipated by Sachdev et al. (1998) *Mol. Cell. Biol.* 18:2524-2534 as evidenced by GenBank Accession No. 1K5JE. The Examiner suggests that Sachdev et al. teach fusion proteins comprising nonclassical NLSs of IκBα, 53BP2, or GABPbeta fused to nucleoplasmin core protein wherein nucleoplasmin core protein contain cationic residues as shown in GenBank Accession No. 1K5JE. Applicant respectfully traverses this rejection.

The reference of Sachdev et al. (1998) teaches that the second akrin repeat in IκBβ and NLSs from 53BP2 or GABPbeta can direct nucleoplasmin core protein to the nucleus, wherein nucleoplasmin core protein is a cationic protein of 124 amino acids in length (GenBank Accession No. 1K5JE). As the specification teaches that the nuclear targeting peptide is conjugated to a small cationic peptide, termed a scaffold (see page 9, lines 2-18), rather than a wholly functional protein as taught by Sachdev et al., claim 7 has been further amended to specify that the scaffold is a peptide of less than 124 amino acids in length. Support for peptides of less than 124 amino acids can be found on page 9, wherein the specification teaches that a scaffold can be 5-200 amino acids in length with specific examples of ~18 amino acids in length provided. Accordingly, Sachdev et al. as evidenced by GenBank Accession No. 1K5JE does not teach each and every element of amended claims 4 and 7, *i.e.* that the NLS interacts with transportin, rather than importin-α or importin-β, to mediate

nuclear pore targeting and import of molecules into the nucleus of cells and, as these references apply to claim 7, they fail to teach a scaffold peptide of less than 124 amino acids in length. Thus, in accordance with MPEP 2131, these references does not anticipate pending claims 4 and 7 under 35 U.S.C. §102 (a) and withdrawal of this rejection is respectfully requested.

Claims 1, 4 and 7 are rejected under 35 U.S.C. §102 (b) as being anticipated by Grondin et al. (1996) *J. Biol. Chem.* 271:15458-15467 as evidenced by GenBank Accession No. I39311. The Examiner suggests that Grondin et al. teach ZNF74 which contains a nonclassical NLS to transport ZNF74 to the nucleus and further contains cationic residues as shown in GenBank Accession No. I39311. Applicant respectfully traverses this rejection.

Grondin et al. teach nuclear localization of ZNF74 and truncated proteins thereof in the range of 334 to 572 amino acids in length which contain a zinc finger region which is responsible for nuclear localization of said proteins. GenBank Accession No. I39311 shows the amino acid sequence of a full-length ZNF74 protein (*i.e.*, 572 amino acids). These references fail to teach each and every element of claims 4 and 7, *i.e.*, they do not teach that the NLS interacts with transportin, rather than importin-α or importin-β, to mediate nuclear pore targeting and import of molecules into the nucleus of cells and, as these references apply to claim 7, they fail to teach a scaffold peptide of less than 124 amino acids in length. Thus, in accordance with MPEP 2131, these references does not anticipate pending claims 4 and 7 under 35 U.S.C. §102 (b) and withdrawal of this rejection is respectfully requested.

Claims 1, 4, 7, and 9-12 are rejected under 35 U.S.C. §102 (b) as being anticipated by Birnstiel et al. (WO 92/13570) as evidenced by Jakel et al. (May 1999) *EMBO J.* 18:2411-23 and Gorlich (1997) *J. Cell Biol.* 138:65-80.

Claims 1, 4, 7, and 9-12 are further rejected under 35 U.S.C. §102 (e) as being anticipated by Birnstiel et al. (U.S. Patent No. 5,922,859, issued 7/13/99, national stage of WO 92/13570) as evidenced by Jakel et al. (May 1999) *EMBO J.* 18:2411-23 and Gorlich (1997) *J. Cell Biol.* 138:65-80.

The Examiner suggests that Birnstiel et al. teach methods for delivering nucleic acids to eukaryotic cells by contacting the cells with compositions comprising a nonclassical NLS covalently linked to a cationic polypeptide, *i.e.*, histone H1. Jakel et al. teach that Histone H1 interacts with importin beta and importin 7 and is considered to have a NLS. As Histone H1 does not bind importin alpha, it is not considered to be a classical NLS because classical NLS bind importin alpha as taught by Gorlich. Applicant respectfully traverses these rejections.

Nowhere in the teachings of Birnstiel et al. is there a teaching of an NLS which interacts with transportin, rather than importin-α or importin-β, to mediate nuclear pore targeting and import of molecules into the nucleus of cells. Accordingly, this reference does not teach each and every element of pending claims 4, 7 and 9-12 and therefore does not anticipate these claims under 35 U.S.C. §102 (b). Withdrawal of this rejection is therefore respectfully requested.

VI. Claim rejections under 35 USC 103

Claims 7 and 9-12 have been rejected under 35 U.S.C. §103 (a) as being unpatentable over Skova et al. (U.S. Patent No. 5,661,025) in view of Michael et al. (1997) *EMBO J.* 16:3587-3598, Michael et al. (1995) *Cell* 82:415-422, or Fan et al. (1998) *Proc. Natl. Acad. Sci. USA* 95:15293-98). The Examiner suggests that the reference of Szoka teaches methods for expressing nucleic acids in eukaryotic cells by contacting the cells with compositions comprising a NLS covalently linked to polylysine complexed with nucleic acids. The Examiner further suggests that while Szoka does not teach nonclassical NLSs, it would have been obvious to one of ordinary skill in the art to substitute the NLS of any one of Michael (1995), Michael (1995) or Fan (1998) for those disclosed in Szoka. It is further suggested that the sequences of Michael (1995), Michael (1997), Fan (1998) and Szoka are all recognized as nuclear localization sequences and would be reasonably expected to function equivalently inasmuch as they deliver attached molecules to the nucleus. Applicant respectfully traverses this rejection.

Applicant respectfully disagrees with the Examiner's suggestion that all nuclear localization signals function equivalently. The present application discloses that transportin-mediated, rather than importin-α or importin-β mediated, nuclear import using a nonclassical NLS is an improvement over the art in that said nonclassical NLS is more efficient in targeting a molecule to the nucleus than using a classical NLS of equivalent charge density and size (see page 12, lines 10-16 of the instant specification). This improvement is particularly advantageous in

gene therapy approaches where more efficient nuclear import of transgenes into the nucleus translates into lower doses and reduced side effects.

To establish a *prima facie* case of obviousness, MPEP 2143 states that three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Nowhere in the primary reference of Szoka is there the suggestion or motivation to substitute an NLS described therein with a nonclassical NLS of any of Michael et al. (1997), Michael (1995) or Fan (1998) to improve nuclear import efficiency via transportin-mediated nuclear import. Further, there is no suggestion or motivation provided in the teachings of the secondary references of Michael (1997), Michael (1995) or Fan (1998) to covalently link a cationic scaffold such as the polylysine of Szoka.

Second, there must be a reasonable expectation of success. While it may have been possible to substitute a classical NLS of Szoka with a nonclassical NLS of any of Michael et al. (1997), Michael (1995) or Fan (1998), there would have been no reasonable expectation that said nonclassical NLSs would interact with transportin, rather than importin-α or importin-β, to transport a molecule into the nucleus of a cell with an efficiency exceeding that of a classical NLS.

Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art and not

based on applicant's disclosure. In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). Whether alone or in combination, the cited references of Michael (1995), Michael (1997), Fan (1998) and Szoka fail to teach the claim limitation of a nonclassical NLS which interacts with transportin, and not importin-α or importin-β, to transport a molecule into the nucleus of a cell.

Thus, when combined, these references fail to establish a *prima facie* case of obviousness as required by MPEP 2143 and therefore withdrawal of this rejection is respectfully requested.

Claim 8 is also rejected under 35 U.S.C. §103 (a) as being unpatentable over Szoka et al. (U.S. Patent No. 5,661,025) in view of Michael et al. (1995) *Cell* 82:415-422 as applied to claim 7 and 9-12 above, and further in view of Beug (US Patent No. 5,354,844) and Formoso et al. (US Patent No. 5,260,189). The Examiner suggests that it would have been obvious to one of ordinary skill in the art to add the sequence GGGC to the C-terminus of SEQ ID NO:1 to facilitate disulfide linkage to a scaffold protein of Szoka et al. conjugated to a NLS of Michael et al. (1995). It is suggested that one would have been motivated to do so because addition of the cysteine facilitates conjugation by disulfide formation as taught by both Beug and Formoso and a glycine spacer preserves function of a conjugated peptide as taught by Formoso. Applicant respectfully traverses this rejection.

As discusses *supra*, the references of Szoka et al. and Michael et al. (1995) alone or in combination fail to establish a *prima facie* case of obviousness with respect to a nuclear targeting peptide containing a nonclassical nuclear localization sequence

which interacts with transportin but does not interact with importin-α or importin-β to import molecules into the nucleus. As the secondary references of Beug and Formoso fail to overcome the deficiencies in the teachings of these primary reference, the combination of Szoka et al. in view of Michael et al. and further in view of Beug and Formoso fail to establish a prima facie case of obviousness as required by MPEP 2143. It is therefore respectfully requested that this rejection be withdrawn.

VII. Conclusion

Applicant believes that the foregoing comprises a full and complete response to the Office Action of record. Accordingly, favorable reconsideration and subsequent allowance of the pending claims is earnestly solicited.

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